PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
39577		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI 2003/000285	14.04.2003	18.04.2002
International Patent Classification (IPC) or national classification and IPC		
C12N 15/11, C12N 15/63, C12N 15/10		
Applicant		
FINNZYMES OY et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.11.2003	21.06.2004
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Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000285

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4)
- ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☒ the international application as originally filed/furnished

☐ the description:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the drawings:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
   - ☒ a sequence listing
   - ☐ table(s) related to the sequence listing

   b. format of material
   - ☒ in written format
   - ☒ in computer readable form

   c. time of filing/furnishing
   - ☒ contained in the international application as filed
   - ☒ filed together with the international application in computer readable form
   - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
   - ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000285

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-13	YES
	Claims		NO
Inventive step (IS)	Claims	1-13	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-13	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:
(Document D7 was not included in the International Search Report but is considered relevant and is therefore included in this report.)

D1) Nucleic Acids Research, Volume 26, No. 8, pages 1927-1933, 1998, York D. et al, "Simple and efficient generation in vitro of nested deletions and inversions: Tn5 intramolecular transposition"
D2) Nucleic Acids Research, Volume 27, No. 13, 1999, pages 2777-2784, Haapa S. et al, "An efficient and accurate integration of mini-Mu transposons in vitro: a general methodology for functional genetic analysis and molecular biology applications"
D3) US5948622
D4) J. Mol. Biol. Volume 314, 2001, pages 433-444, Lee I. et al, "Importance of the Conserved CA Dinucleotide at Mu Termini"
D5) Journal of Virology, Volume 74, No. 6, 2000, pages 2760-2769, Chang Laurent L. et al, "Functional Characterization of the Human Immunodeficiency Virus Type 1 Genome by Genetic Footprinting"
D6) Trends in Microbiology, Volume 8, No. 12, December 2000, pages 571-577, Hayes F. et al, "Pentapeptide scanning mutagenesis: encouraging old proteins to execute unusual tricks"
D7) J. Mol. Biol. Volume 310, 2001, pages 299-309, Coros and Chaconas, "Effect of mutations in the Mu-host junction region on transposome assembly"

D1 shows a transposition deletion vector used in a method for

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

the generation of nested families of deletions. A Tn5 in vitro transposition system is used. Immediately adjacent to each end of the transposon end sequences are three stop codons in all three reading frames. The positioning of the stop codons leads to formation of C-terminally truncated proteins for structure-function deletion analysis of any protein. Markers for selection or screening are included in the transposon construct. The proteins produced from various deletions were found to be of the expected molecular weight, as determined by SDS-PAGE. See especially p. 1930 right column paragraph 3 and p. 1932 right column paragraph 2.

D2 shows a Mu-derived transposition tool that can be used in functional genetic analysis. It is stated that any DNA segment attached between Mu ends can be used as a mini transposon and that transposons aimed at protein engineering applications will be especially valuable. These include additions into the gene of interest of sequences coding for sequence tags or translation stop codons. See p. 2781 left column paragraph2 lines 1-3, p. 2781 right column last paragraph and p. 2782 right column-p. 2783.

D3 shows a Tn5 based transposition system. Polypeptides having amino- or carboxy-end truncations can be produced for analysing relationships between protein structure and function. Stop codons are included in the transposon construct adjacent to the OE termini for terminating translation of a coding sequence present on the plasmid. Selectable markers or tags can be included in the construct. Mutations in the transposase are discussed and acceptable termini modifications are screened. Mutant transposon end sequences resulting in hyperactive transposons with an increased in vivo transposition frequency are shown. It is stated that the transposon can be used to introduce a desired restriction enzyme site. See col. 2 lines 32-26, col. 3 lines 26-31, col. 4 lines 58-65, col. 5 lines 4-15, col. 9 lines 15-60, col. 13 lines 44-60 and col. 23 line 46- col. 24 line 5.

D4 shows that the two last base pairs in the termini of

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

transposable phage Mu can be modified without severe effect on transposome function. The activity of all 16 dinucleotide permutations of the termini is examined. See the abstract and p. 422 left column paragraph 2.

D5 shows the production of a transposon containing a NotI restriction site close to the transposon end, resulting in a changed base pair in the R1 sequence. A library of insertion mutants is constructed in vitro using the MuA transposase.

D6 shows a transposon-based scanning linker mutagenesis method for dissecting protein activity and function. Random insertion of a variable five amino acid cassette into a target protein is achieved.

In D7, a series of mutations at the Mu-host junction are examined in relation to the Mu in vitro strand transfer reaction. The degree of inhibition is demonstrated to be dependent upon the particular base-pair change at each position and whether the substitution occurs at the left or right transposon end. The conserved AC at each end as well as one nucleotide on each side of the Mu-host junction are mutated individually and their effects on the in vitro reaction is examined. See the abstract and p. 301 figure 2.

The present application relates to methods and materials for producing C-terminal deletion derivatives of polypeptide encoding nucleic acids, by the use of a modified transposon with stop codons in all three reading frames, said codons being at least partly within the transposon end sequence recognised and bound by a transposase, which catalyse the transposition.
The problem to be solved is to generate a number of deletion derivatives simultaneously and with ease. The solution is achieved by placing stop codons at least partly within the transposon end, which strategy leaves at its best no extra amino acid residues in the C-terminus of the deletion derivative. Extra amino acid residues may interfere with the protein function and are therefore not desirable.

D1 is considered to represent the closest prior art. The deletion derivatives produced in D1 are stated to be of the

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

expected molecular weight.

The difference between the invention according to claim 1 and D1 is that in the present application, the stop signals are at least partly positioned within the end sequence of the transposon. In D1, the stop signals are immediately adjacent to the end sequence. In deletion derivatives of D1, the distance between the actual deletion site and the first stop codon is always at lest 19 nucleotides (the length of the Tn5 transposon end is 19bp), which results in the addition of extra amino acid residues attached in the C-terminus. The strategy of D1 always leaves a tail of 7-9 amino acids (deduced by counting different reading frames) and the sequence of the tail varies in different reading frames. The "expected size" of D1 always comprises the extra tail.

In the present invention a pool of deletion derivatives with no extra amino acid translated from the transposon end sequence or maximally a tail of 2-3 amino acids (the length of the tail being dependent on the reading frame) are produced.

Thus, there is a technical effect over D1 since the extra tail is absent or at least significantly reduced. Thereto, if needed, tailless derivatives can be selected from the pool (a possibility that is not mentioned in D1), which renders possible an effective functional study of target protein without method-born uncertainties.

Consequently, the problem to be solved by the invention according to claim 1 is the provision of deletion derivatives with no, or significantly reduced, extra amino acid tail. The problem to be solved by the invention according to independent claim 9 is to provide a method for producing such deletion derivatives.

D3 discloses point mutations with positive effect on transposition frequency. The document does not give any indication which nucleotides are essential for the function of the transposon and the teachings of D3 can not be used to conclude which areas of the conserved transposon end, if any, that would tolerate a series of adjacent point mutations.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D4 discloses modification of the conserved CA dinucleotide at Mu termini. However, said conserved CA dinucleotide does not reside in Mu transposon end sequences. Thus, D4 does not teach anything about mutation tolerance of R1 or R2 sequence.

In D7, the effect of mutations at the distal ends of the native Mu transposon is disclosed. The modifications are located at or near the same CA dinucleotide as disclosed in D4. No modifications to R1, R2 or corresponding L2 sequences are shown.

To create translational stop signals in three reading frames, to be located partly within the transposon end sequence recognised by a transposase, the skilled person would have to consider modifying several and most likely adjacent nucleotides of said transposon end.

D5 discloses an engineered site, a NotI site, close to the transposon end. However, no adjacent nucleotides are modified in the R1 sequence. Other prior art is silent on engineering a predeterminate site to the transposon end.

D2 can even be considered to teach away from the present invention by disclosing that functional fragments should be attached between the transposon ends (see D2 p. 2782, right hand col. last paragraph, last sentence). No other solutions are suggested.

There are certain prior art documents showing that mutations within R1 and R2 sequences have negative effect on transposition efficiency. However, generally these documents are old.

Thus the skilled person would faced with the problem of providing deletion derivatives with no, or significantly reduced, extra amino acid tail, would not be motivated by the prior art to consider altering the transposon end as to create the transposon nucleic acid according to claim 1.

Thus, the cited prior art does not give any indication that

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

would lead a person skilled in the art to the claimed transposon nucleic acid or the claimed method for producing a deletion derivative. Therefore, the claimed invention is not obvious to a person skilled in the art.
Accordingly, the invention defined in claims 1-13 is novel and is considered to involve an inventive step. The invention is industrially applicable.